EXPERIMENTAL CEREBRO-SPINAL MENINGITIS IN MONKEYS.

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PLATES II–V.

At the beginning of my study of *Diplococcus intracellularis* I had the plan of using monkeys to reproduce the symptoms and lesions of cerebro-spinal meningitis in man. My study of the intraperitoneal injections of guinea-pigs encouraged me to believe that the injection of suitable cultures of the intracellularis into the spinal canal of monkeys would lead to the production of an acute inflammation, the symptoms and lesions of which might bear resemblance to those occurring in the natural infection in human subjects. I was not mistaken in this belief. Some of my earliest experiments were made with monkeys. The cultures were introduced into the spinal canal by means of lumbar puncture. The injection was made commonly in the canal at the level of the third lumbar space. This space is not below the level of the cord in many monkeys, but I have never had paralysis follow my inoculations. I endeavored to secure a flow of spinal fluid through the needle before injecting the culture, for, otherwise, doubts whether the injection was carried into the canal or the surrounding muscles might arise. Not infrequently I failed to obtain this flow of fluid even when I felt reasonably certain that the needle was in the canal. In endeavoring to remove this source of doubt I introduced, in a few instances, the needle into the fourth and fifth lumbar spaces, without causing, in a single instance, paralysis. It sometimes happened that fluid could be obtained at these higher levels when it was not secured at the lower ones. Small monkeys, as a rule, seem not to have any considerable quantity of free fluid in the spinal canal. In this respect, this class of animals differs greatly from man. The amount of fluid which is obtainable by lumbar puncture from the monkey

is subject to wide variation in the same species, and in different species. The amount which may be secured from an animal on successive days also differs greatly. In some cases, a few drops may be secured after patient waiting for five or ten minutes; in other cases, the flow sets in immediately and one or two cubic centimeters are rapidly obtained. As will appear below, the quantity of fluid is increased at certain stages of the inflammatory process, to diminish or disappear wholly at others.

In cases in which no spinal fluid has been obtained preceding the injection of the culture, a successful injection can be assumed if the monkey develops symptoms of illness within a few hours of the inoculation, or if a subsequent puncture of the spinal canal yields fluid containing the diplococci and exuded inflammatory cells. I have found that the injection of relatively large quantities of living diplococci into the muscles about the spinal column, or into the muscles and subcutaneous tissues of other parts of the body, produces only trifling effects. There appears at most some local swelling, and the animals may refuse food for a few hours. The tumefaction quickly disappears and the appetite returns. There is a parallel in this respect between the relative susceptibility of the guinea-pig to intraperitoneal inoculation and the high degree of refractoriness which it displays toward subcutaneous injections of the diplococcus. Not a few cultures of the diplococcus have failed to produce symptoms or lesions when injected into the spinal canal of monkeys. Virulence plays an important part in these injections. In some instances the degree of virulence as exhibited in monkeys and guinea-pigs was similar. Cultures which were active against guinea-pigs were active in monkeys; a culture of low virulence for the former was little pathogenic for the latter animals. This parallelism was well shown by a culture "Smith," which retained its virulence through several months of artificial cultivation. When its power to infect guinea-pigs was finally greatly diminished, it had lost to a large degree its power to cause serious lesions in monkeys. In the experiments upon monkeys to be related, suitable cultures for inoculation were, as a rule, chosen by testing them in advance upon small guinea-pigs.

At the time my experiments were begun there was, as far as I

was aware, only one report in the literature upon the use of monkeys to produce cerebro-spinal meningitis by injecting cultures of *Diplococcus intracellularis* into the central nervous system. Bettencourt and Franca[1] attempted without success to infect monkeys. They trephined one monkey and introduced the culture beneath the dura mater; they injected in two other monkeys cultures into the spinal canal. In still another monkey they attempted to infect the animal by rubbing the nasal mucous membrane with cotton moistened with a culture of the intracellularis. The successful experiments of Von Lingelsheim and Leuchs[2] were published after my first communication on this subject had appeared.[3] Weichselbaum[4] succeeded by subdural inoculation in three instances in dogs in producing acute pachy- and leptomeningitis associated with encephalitis; Councilman, Mallory and Wright[5] successfully produced acute meningitis in a goat by injecting a bouillon suspension of the diplococcus into the spinal canal. Albrecht and Ghon,[6] and Bettencourt and Franca[7] failed to confirm this result in their experiments upon goats.

The monkey offered itself as a suitable animal for the inoculation experiments because of its relationship to man; the fact of its upright carriage, which would permit comparison with man in respect to the distribution of the lesions, and because of the ease and safety with which repeated lumbar puncture could be made in following the course of the disease. The protocols of certain successful experiments will be given.

Monkey No. 1. Macacus Nemestrinus.—April 12, 1905, 10:30 A. M. A moderately large monkey was etherized,[8] after which a needle was introduced into

[1] Uber die Meningitis Cerebro-spinalis Epidemica und ihren specifischen Erreger. *Zeit. f. Hyg. u. Infectionskrank.*, 1904, xlvi, 463.

[2] Tierversache mit dem Diplococcus intracellularis (Meinigococcus), *Klin. Jahrbuch*, 1906, xv, 489.

[3] Experimental Cerebro-spinal Meningitis and its Serum Treatment, *Jour. Amer. Med. Assoc.*, 1906, xlvii, 560.

[4] *Fortschritte der Medicin*, 1887, v, 622.

[5] Epidemic Cerebro-spinal Meningitis and its relation to other forms of Meningitis, Boston, 1898.

[6] *Wien. klin. Woch.*, 1901, xiv, 984.

[7] *Op. cit.*, page 500.

[8] In later experiments ether was not used, as the operation gave little pain.

the spinal canal. Clear fluid escaped from the needle. A culture eighteen hours old of *Diplococcus intracellularis,* in its first generations on Loeffler's serum, suspended in salt solution, was injected. The monkey quickly recovered from the operation. Six hours later it appeared to be sick; at 6 P. M., it no longer sat on its perch; 9 P. M., still on bottom of cage, very sick, head down. On being disturbed, it moved slowly. Death during the night. Survived injection probably eighteen to twenty hours.

Autopsy 9 A. M., April 13. The internal organs, except the nervous system, show no striking lesions. Brain and Spinal Cord.—No marked excess of fluid in the meninges. The convex surface of the brain is greatly congested, and punctiform hæmorrhages exist in the pia-arachnoid and the superficial portions of the cortex. No definite exudate can be made out on the convex surface of the convolutions, although the pia of the sulci appears white and is slightly thickened (probably an old condition). A focus of softening or an abscess, the size of a large pea, occurs to the right of the superior longitudinal sinus in the convolutions anterior to the motor area, and just beneath the membranes. The base of the brain is covered with an opaque exudate, white in color, which extends over the medulla and, anteriorly, to the optic commissure. The lumbar and thoracic portions of the spinal cord are covered, chiefly posteriorly, with an opaque white exudate. The cerebral ventricles contain an increased amount of fluid of turbid appearance.

The diplococcus was recovered in cultures from the exudates of the spinal cord and base of the brain, and from the blood of the heart. Cover-glass preparations from the exudates show that (1) the pia of the convexity contains many polymorphonuclear leucocytes and few diplococci; (2) the pia covering the medulla contains large numbers of leucocytes and intracellular cocci, and a small number of extra-cellular cocci; (3) the ventricles contain desquamated ependymal epithelium, many leucocytes, and few diplococci; and (4) the focal lesion in the convexity of the brain consists of a collection of polymorphonuclear leucocytes containing very large numbers of typical diplococci. Few or no diplococci occur outside of cells in this focus (Plate II, Fig. 1).

Sections of the brain and cord show an abundant emigration of polymorphonuclear leucocytes into the leptomeninges chiefly. The pia-arachnoid of the convexity contains a richer exudate than was evident to the naked eye; but the exudate covering the base of the brain is present in much the greater amount. It consists chiefly of leucocytes; fibrin occurs in very fine strands and in small amount only. The ventricular fluid contains many leucocytes and no fibrin. A striking feature of the inflammation is the invasion from the pia-arachnoid of the superficial portion of the cortex directly, and of removed parts at considerable depths indirectly along the sheaths of the blood vessels. The vascular sheaths and the perivascular lymphatic spaces are richly infiltrated with polymorphonuclear leucocytes from which locations the surrounding brain tissue is being invaded by emigrating leucocytes. Sections through the filum terminale show the leucocytes to surround the cord in the pia, a slight invasion along the septa into the nervous tissue, and thick perivascular emigration about the deepest vessels of the dura mater.

This successful experiments was obtained by the use of a recent

culture isolated from the spinal fluid of a child "Kepp." The fluid was quite opaque from the many leucocytes present and it contained a moderate number of the diplococcus, both within leucocytes and free in the fluid. A second monkey of the same species and about the same size as the first was inoculated with one cubic centimeter of the sedimented exudate. Immediately after the injection, the monkey became rigid and the head was retracted; recovery took place in a short time. In spite of the fact that no fluid escaped from the needle, no doubt was entertained that the injection entered the spinal canal. However, no symptoms of disease developed from this injection.

Monkey No. 3. Small Macacus rhesus.—April 15, 1905, 9. A. M. Inoculated by lumbar puncture with two loops suspended in salt solution of "Bingley" culture of the diplococcus from spinal fluid. 5 P. M., animal sick; sits on bottom of cage. 9 P. M., very sick; crouches on bottom of cage with head depressed; moves slowly on being disturbed. April 16, 9 A. M., still alive; head down almost to level of the floor; a little later, the monkey is lying on one side and is passing through a convulsion. The convulsive seizures follow each other at short intervals and are excited by sudden noise or by contact. 3 P. M., convulsions still occurring; lumbar puncture yields a small quantity of bloody fluid which on microscopical examination shows many polynuclear leucocytes containing typical diplococci. 10 P. M., convulsions continue. Animal died during the night; probably survived about forty-three hours.

Autopsy, 9 A. M., April 17. No visible lesions of the internal organs except the central nervous system. The convex surface of the brain shows great injection of the pial vessels; and the pia-arachnoid contains a gelatinous fluid exudate. Purulent exudate is visible surrounding the infundibulum only. The fluid in the ventricles is slightly increased. No visible exudate covers the spinal cord. The cultures made from different portions of the brain and cord remained sterile. Sections of the spinal cord show a small quantity of purulent exudate in the meninges; those from the brain show a richer leucocytic infiltration of the pia-arachnoid, and the invasion of the choroid plexus of the lateral ventricles. The meninges of the sulci are especially infiltrated. From the superficial meninges, and from the intra-cortical vessels, the brain substance has been infiltrated with many leucocytes.

As regards this experiment I wish to point out that when the inoculated monkey survives through the second day, the quantity of exudate may not be very considerable, and the diplococci, if still present, may fail to grow on a suitable culture medium.

Monkey No. 7. South American; Genus Cebus.—May 19, 1905, 3 P. M. One cubic centimeter of a suspension of spinal fluid culture "Goldman" injected. This South American species is considerably smaller than the Macacus monkeys employed. It was found dead at 7 A. M., May 20, but evidently had been dead

only a short time. Probably survived fifteen hours. The autopsy showed a small amount of exudate over the spinal cord in the lumbar region, turbid fluid along the base of the brain to the optic commissure, vivid injection of the pia of the convexity, and an increased quantity of turbid fluid in the cerebral ventricles. Cultures were positive. Smear preparations from different parts of the brain and cord show the leucocytic exudate to be general, and a fairly large number of diplococci, chiefly intracellular, to be present. Many of the diplococci within leucocytes are swollen or otherwise degenerating. The preparations made by smearing bits of the nasal mucosa on slides show a considerable number of polymorphonuclear leucocytes among the high epithelium and mucus. *A small number of the leucocytes contain many diplococci morphologically like those of the exudate in the brain, and presenting the same degenerations.*

A study of the brain and cord in sections shows the inflammatory lesions over the convexity to be more pronounced than was evident to the naked eye. The pia is everywhere invaded by polynuclear cells, and the exudations form thick, wedge-shaped infiltrations between the convolutions. A fine network of fibrin unites the leucocytes. The invasion of the cortex is a marked feature of the lesions. The leucocytes have passed into the brain tissue from the surface and along the sheaths of the vessels (Plate II, Fig. 2). An intermediate zone of non-infiltrated tissue exists between the two layers. The invasion of leucocytes into the brain stops rather abruptly a centimeter or so from the surface, and the deeper vascular sheaths are devoid of these cells. Among the leucocytes which have emigrated into the cerebral tissue are a small number of eosinophilic cells. A section through the medulla shows less exudate than some parts of the cortex. The deeper parts of the medulla have also been invaded from the vascular sheaths. The lateral ventricle has been invaded along the choroid plexus, and an accumulation of leucocytes occurs below the epithelium, which is partly deficient, at one side of the ventricle. The thoracic region of the spinal cord is relatively free from exudate.

A spinal root ganglion included in a section of the spinal cord is surrounded, beneath its fibrous capsule, by a collar of leucocytes which are penetrating among the nerve cells (Plate III, Fig. 3). Sections of the optic nerve show leucocytic invasion. Diplococci are numerous in the leucocytes in the pia-arachnoid. They cannot be found with certainty in the leucocytes or free in the brain tissue.

This experiment serves to show that in a brief period of fourteen or fifteen hours advanced and deep lesions can be produced in the brain and its membranes by *Diplococcus intracellularis*. The experiment also indicates that the inflammatory reaction may be more active in the brain than in the spinal cord. The inflammatory exudate resembles in character that present in acute cases of epidemic meningitis in human subjects. Emphasis should, perhaps, be laid upon the findings in the nose and in the spinal ganglion. It is usual to find in the monkeys succumbing to the experimental infections, evidences of inflammation of the nasal mucosa. Smear preparations from the superior mucous membrane often show a

variable number of leucocytes enclosing diplococci presenting the morphology and staining properties and the degenerations of the diplococci in the brain and cord. The inflammation and purulent infiltration of the spinal ganglion is interesting in relation to the similar finding in cases of cerebro-spinal meningitis in man by Councilman, Mallory and Wright.[9]

Monkey No. 10. Macacus rhesus.—May 26, 1905. At 11 A. M. one half agar culture "Cohn" suspended in salt solution was injected into the spinal canal. Fluid escaped from the inserted needle. 5 P. M., animal very sick; living at 9 P. M. Found dead at 6 A. M., May 27. Probably survived fifteen hours.

Autopsy: The meninges of the inoculated portion of the cord show minute hæmorrhages and cloudy exudate. The meninges of the medulla contain an increased quantity of cloudy fluid. The pia covering the convex surface of the brain is hyperæmic, especially over the occipital lobes, which present an almost uniformly reddish tint; while the pia and the adjacent brain substance are everywhere beset with minute hæmorrhages. Cultures from the lumbar portion of the cord, third ventricle, and pia over the medulla all give pure growths of the diplococcus. No growths are obtained from the cortical meninges and the lateral ventricle. The furthermost portion of the dura mater, extending beyond the olfactory lobes into the nose, is covered with an inflammatory exudate in which polymorphonuclear leucocytes carrying biscuit shaped Gram-negative diplococci exactly resembling in form, preservation and staining, those present in the meninges of the brain and cord occur. Smear-preparations from the turbinated bones show very few leucocytes and no diplococci. Cultures made from the mucus of the turbinated mucous membrane did not yield the diplococcus.

The sections of the brain show a moderate inflammatory œdema affecting the pia-arachnoid of the base and the convexity. The especial pathological conditions to be emphasized are (*a*) the great congestion of the pial blood-vessels and the extravasation of red corpuscles; (*b*) the direct inflammatory invasion of the superficial cortex from the pia which is associated with rarification of the brain tissue; (*c*) the profound changes in the blood-vessels of the brain proper and the degeneration and leucocytic invasion of the surrounding brain tissue (Plate III, Fig. 4). In some parts of the cortex, adjacent to the sulci, the leucocytic accumulation is so rich as to simulate abscess formation; no softening has, however, taken place. The emigration of leucocytes from the intracortical vessels was active at the time of fixation of the tissues. The perivascular lymphatics contain fibrin and coalesced red-corpuscles, and the blood-vessels themselves are occluded, in places, by coalesced (agglutinated) red corpuscular and by fibrinous thrombi. The punctiform hemorrhages in the brain tissue arise from these injured and occluded vessels. *Sections of the dura mater extending beyond the olfactory lobes show a richer accumulation of leucocytes than elsewhere in the membrane itself and an invasion of the substance of the membrane.*

Typical diplococci occur within leucocytes in the meningeal exudation and

[9] *Op. cit.*, p. 114.

they are moderately numerous. They exist also in the leucocytes in the brain tissue, adjacent to the blood-vessels from which the leucocytes have emigrated. No diplococci are found in the brain tissue outside of cells.

A lengthy discussion of this experiment is not called for since it carries its own explanation. Attention may, perhaps, be directed again to the co-incident effects produced by the diplococcus upon the membranes and the nervous tissues. The wide involvement of the blood vessels is a significant fact in the pathology of this experimental disease; and the evidence of diffusion of the poison from the blood vessels of the brain or from the peri-vascular lymphatics into the brain tissue is made strong by this experiment. The agglutinative red corpuscular thrombi constitute a novel feature of the pathological condition. The rich assemblage of leucocytes surrounding the olfactory lobes and extending into the substance of the dura mater seems to me not wholly without significance in view of the possibility of infection of the nasal passages with diplococci from the brain.

In view of the importance of this condition for explaining the appearance at times, of *Diplococcus intracellularis* in the nasal and pharyngeal cavities in human subjects of cerebro-spinal meningitis, I gave especial attention to the study of the olfactory nerves and the nasal passages in several monkeys. It may, in the first place, be stated that the turbinate and septal mucous membrane will be found to be vividly congested in all the animals which succumb to the acute infection. If smear preparations from the mucous covering the inflamed membranes, or, better, from bits of the mucous membrane snipped off with scissors be examined, a variable but increased number of polynuclear leucocytes will be seen. If the leucocytes are compared with those present in the meninges about the base of the brain, they will be found in the same condition of preservation or degeneration as the latter. Moreover, in those cases in which diplococci are still demonstrable in the leucocytes in the brain, a certain number, sometimes relatively many, of the leucocytes in the nose contain Gram-negative diplococci resembling in all external features the former micro-organisms. If the dura mater beginning at the olfactory bulbs, surrounding the olfactory nerves, and extending through the cribriform plate into the nose, be carefully removed with the adjacent

portion of the ethmoid bone and olfactory mucous membrane, fixed in Zenker's fluid, sectioned longitudinally, stained in hæmatoxylin or methylene blue and eosin, and examined microscopically, the passage of leucocytes from the brain cavity into this membrane and about the olfactory nerves towards the ethmoid, can be traced. The blood-vessels of the adjacent olfactory mucous membrane are seen to be dilated and leucocytes to be passing into the layer of columnar epithelium of the surface.

The abundant lymphatics of the mucous membrane are in communication with the lymphatic spaces which enclose the branches of the olfactory nerves, and these spaces again communicate with the subdural and subarachnoid spaces of the cranium, so that the lymphatics of the nasal mucous membrane can be injected, according to Schwalbe and Key and Retzius, from the cranial cavity.

I have not succeeded, although I have made several attempts, in cultivating *Diplococcus intracellularis* from the nasal mucosa of these monkeys. I realize, of course, that it is highly desirable and very important to bring this final proof of the passage of the diplococci from the cranial cavity into the nose. The difficulties which surround the demonstration are considerable, for I think it probable that the diplococci do not long remain viable in the nose in monkeys. I conceive the conditions there to be, possibly, even more unfavorable to them than in the meninges where, indeed, they tend rather to disintegrate than to multiply. The conditions in man are, of course, very different and far more favorable to the existance of the diplococci. But in spite of the incompleteness of the proof in the case of the monkeys, I would still urge that attention be given to this possible mode of infection of the nasal and pharyngeal passages in human subjects.

The next experiment to be described brings out a different point, namely, that of a fatal issue, after the lapse of thirty hours, with which is associated the gradual diminution and degeneration of the diplococcus, without any marked participation of leucocytes in the process, or production of striking pathological lesions. It recalls forcibly certain observations made on guinea-pigs.

Monkey No. 12. South American; Genus Cebus.—June 1, 1905, at 11 A. M., a small monkey was inoculated with one half agar slant culture "Smith"; fluid

flowed from the needle before injection. 12 M., lumbar puncture (= l. p.) 1 c.c. almost pure blood obtained; it showed on cover-slips (= c. s.) many free diplococci. 3:15 P. M., l. p. small quantity blood secured; c. s. many cocci free and very few in leucocytes. Animal not sick. 9 P. M., l. p. a few drops of slightly blood-tinted fluid obtained; c. s. many free diplococci and a small, although increased number of leucocytes containing diplococci. Animal less lively than before. June 2, 9 A. M., monkey is very sick; temperature subnormal; head droops below arms; no convulsions on disturbance; l. p. a few drops of blood-tinted fluid secured; c. s. leucocytes increased, some containing diplococci part of which stain feebly; many free diplococci. 3 P. M., l. p. leucocytes still not numerous; intracellular and extracellular diplococci present, but the number is diminishing. Cultures from the fluids obtained at the punctures gave growths in every instance. About 4 P. M. the monkey showed general weakness and retraction of the head. Dr. Meltzer, who examined the animal, pronounced the latter condition not to be opisthotonus. Death at 5 P. M. On ice till next day.

Autopsy, June 3, 9 A. M. Neither the cord nor the brain show any marked lesions.

The pia of the cord is slightly injected; a small quantity of clear fluid is present in the pia of the medulla; the intraventricular fluid may be slightly increased. Cultures are negative from brain and cord and the heart.

Smear preparations from the meninges of cord and brain and the fluid of the ventricles show a small or moderate number of diplococci lying free, and almost no leucocytes whatever except in the spinal cord at the point of inoculation. A moderate number of the cells containing diplococci, many of which are degenerating, occur there. Elsewhere it is exceptional to find polynuclear cells containing diplococci. The failure of the diplococcus to grow in cultures made at autopsy does not signify, since the power to grow may be quickly lost at the temperature of the refrigerator. Smear preparations from the nose show no typical diplococci, while a few typical organisms are present in the smears from the pia about the olfactory lobes. The microscopical examination of sections of the spinal cord and brain bear out the naked eye appearances. The membranes show a slight but definite accumulation of leucocytes; there is no œdema, and the nervous tissue is free from appearances of degeneration or from leucocytic invasion. The leucocytes are, however, increased in numbers in the cortical vessels.

The next experiment records an instance of fatal effects, from a relatively small dose of the diplococcus, which was delayed until about sixty hours after injection. The gross lesions were slight; the microscopical ones were definite and interesting because the period of infection was long enough to permit of reaction on part of the fixed tissue cells.

Monkey No. 18. Macacus rhesus.—June 24, 1905, 10 A. M., injected one third culture "Smith." Symptoms of illness developed in the usual time and were pronounced during the first twenty-four-hours. June 25, 12 M., lumbar puncture. The fluid obtained showed many leucocytes, but no diplococci. Cul-

ture negative. The animal did not fully recover and died about sixty hours after inoculation. The autopsy revealed congestion of the meninges, and a small excess of turbid fluid in the lateral ventricles. Cultures on sheep-serum agar were made from several parts of the nervous system. Growths were obtained on the second day, starting from the condensation water, in the tubes inoculated from the third ventricle and lumbar cord. Sections from the brain and cord show, first, a marked congestion of the veins which is greater in the meninges of the brain than of the cord. Second, a small degree of leucocytic exudation throughout the meninges, which in the brain is more abundant in the sulci than over the convolutions. But the most striking and, perhaps, important feature is the great increase of large monoculear pial or connective-tissue cells which by reason of their number make up a large part of the exudate. These cells are larger than leucocytes, possess single vesicular nuclei excentrically placed, and pale and transparent protoplasm.

To be contrasted with the previous experiment is the next one in which the inoculated monkey survived hardly more than twelve hours, in the course of which period marked gross lesions appeared in the nervous system.

Monkey No. 17. South American; Genus Cebus.—June 22, 1905, 10 A. M., injected one half agar culture "Smith," twenty-four hours old. Fluid flowed from needle before injection. 9 P. M., monkey dying. Found dead at 6 A. M., June 23. The autopsy showed the entire spinal cord congested and infiltrated with turbid fluid. In the cerebral meninges smaller and larger hæmorrhagic foci were visible. The pia of the pons and medulla was vividly congested, and a fluid exudate occupied the meshes of the pia-arachnoid generally, and was most abundant over the medulla. The ventricles were unchanged, apparently. Cultures gave the following result: Abundant growth of the diplococcus from the lumbar cord and base of brain; a few discrete colonies from the cortex; no growth from the lateral ventricle. Smear preparations bore out the naked eye indications of the distribution of exudate and diplococci. Sections of the tissues show marked leucocytic infiltration of the membranes of the cord and brain, and a greater accumulation of cells in the basal membranes and the membranes of the sulci. The infiltration is wholly polymorpho-leucocytic. The most striking lesion, however, is the hæmorrhage which is focalized in the membranes and in the superficial brain matter—in the basal and cortical parts. The hæmorrhages in the brain tissue proper have arisen (*a*) by direct extension from the menigeal extravasations, and (*b*) from the vascular branches within the substance of the organ itself. They form elongated or circular foci, depending on the direction of the section. Another lesion of interest is an acute endarteritis which affects the larger and smaller arteries, chiefly at the base of the brain. A sub-intimal infiltration of cells consisting of polymorphonuclear leucocytes and mono-nuclear cells is seen. The vessels are rarely wholly or almost occluded by this accumulation of cells beneath the intact endothelium. Acute encephalitis was a prominent feature of this case.

This experiment, taken into account with others to be described, tends to show that the diplococci rise in the spinal canal to the

medulla, spread themselves over the base of the brain, extend into the cortical meninges, and lastly enter the ventricles. The inflammatory lesions follow in the wake of this extension and doubtless are directly due to the presence of the diplococcus. Attention should perhaps be directed to the hæmorrhages; and to the acute endarteritis mentioned in this protocol of which other examples will be described.

Monkey No. 25. Macacus rhesus.—June 21, 1906, 11 A. M. Inoculated with one agar culture "596." Fluid flowed from needle before injection. 2 P. M., animal appeared sick; lay on bottom of cage, but on being disturbed sat up. 6 P. M., depression increased. 12 P. M., still lying on bottom of cage, but not prone and rises to sitting posture when disturbed. June 28, 8 A. M., brighter; sits up. 12 M., attempted lumbar puncture. Animal resisted with considerable vigor making the puncture difficult. The struggles brought on sudden collapse from which partial recovery took place. Monkey lay on bottom of cage breathing very rapidly; drank water. 2 P. M., still very sick. From this time until 3:45 P. M., when death occurred the animal became weaker and developed intermittent convulsions. The autopsy showed no general visceral lesions. On exposing the spinal cord, it was found congested and covered with a thin, whitish exudate which was more abundant in the lumbar region than in the thoracic and cervical regions. The blood-vessels of the dura mater were injected; but the turgid pia vessels showed through this membrane. On removing the dura, the pia-arachnoid was seen to be vividly injected, the congestion affecting the main and smaller vascular branches and producing a remarkable picture of hyperæmia. There was no visible exudate over the convex surface of the brain. At the base, extending from medulla to optic commissure, there was a turbid fluid exudate. The ventricles contained turbid fluid, but were not dilated. The basal exudate followed the dura over the olfactory lobes into the ethmoid plate. The nasal mucosa was congested. Cover-slips showed the following: From the lumbar spinal cord many leucocytes and diplococci, the latter both free and in cells; from the thoracic and cervical regions of the cord, many leucocytes and fewer cocci; from the convexity—anterior and posterior—impression preparations indicated that few or no leucocytes collected in this region, and the diplococcus was almost wholly absent; from the medulla many leucocytes and extra- and intracellular diplococci; from the ventricles leucocytes and diplococci in small numbers; from the deepest part of the dura at the ethmoid bone many polynuclear leucocytes, but no typical examples of the diplococcus. Cultures from all the sources remained sterile. Since more than two days elapsed, owing to accidental circumstances, between the death of the monkey and the autopsy, it may well be that the failure of cultures was due to this cause. The same condition is capable of diminishing the number of diplococci which can be made out in smear-preparations. But the distribution of emigrated leucocytes showed clearly where the infection was severe, and where it was in its beginning stages. The sections of the tissues bear out the gross findings. They show the existence of great congestion of the pial veins, a small degree of leucocytic infiltration of the pia covering the con-

volutions, and somewhat greater accumulation of inflammatory cells in the sulci. The pia of the deeper parts of the sulci is hardly more than œdematous. The brain substance shows no leucocytic invasion, and the veins do not carry any marked excess of leucocytes. The lower spinal cord shows rather more emigration of leucocytes than the brain. The exudate is collected on the surface of the cord, upon and between the nerve roots (Plate IV, Fig. 5). Fibrin occurs as fine strands and in small quantity.

This experiment brings out several points, some of which have not been especially emphasized before. The effects of muscular exertion on the progress and termination of the infection should, perhaps, be remarked. Since in no other experiment was so great a degree of vascular congestion noted, I should be inclined to view this condition as one of the striking consequences of the muscular over-exertion. It is a short step from this assumption to supposing the marked œdema of the pia in the sulci to have followed the congestion. This view is rather borne out by the poverty of the œdematous fluid in emigrated cells. It has been pointed out on the basis of some of the earlier experiments, and the same fact is indicated by this experiment, that the injected diplococci tend, regularly, to be distributed upwards along the spinal cord and the base of the brain. The invasion of the pia of the convex and mesial surfaces of the brain takes place at a later period, and the infection of the ventricles also takes place later, either because the diplococci are delayed in reaching them, or because they resist infection longer. There is, moreover, relation between localization of the diplococci and the degree and extent of the exudation. Where the accumulation of inflammatory products is most marked the diplococci will, if still present at all, be most abundant. Finally, the growth of the diplococcus upon a suitable culture medium will be greatly influenced, if not wholly determined, by the conditions to which the infected body is subjected after death. While a temperature just above the freezing point will preserve the morphological elements of the body and even the body of the diplococcus, yet if it is maintained for many hours, it may render the diplococcus incapable of multiplication. Higher temperatures subject the body not only to autolytic and putrefactive processes which interfere with the pathological examination, but they deprive even more quickly than cold the diplococcus of power of growth and, under certain conditions, of staining properties as well.

The temperature at which the body is kept, and the elapsed period of time between death and the examination, have a direct bearing upon the cultivation of *Diplococcus intracellularis* from the infected body. I am of the opinion that these factors operate in much the same way in cases of human meningitis as in the case of the artificially infected monkeys and guinea-pigs. Certain discrepancies in the bacteriological studies of cases of meningitis in man may, in view of these considerations, receive an explanation.[10]

On account of its bearing upon this subject and as a transition to the next topic to be discussed, an experiment in which the influence of two extremes of temperature upon the diplococcus was tested, will be introduced here.

Monkey No. 36. Macacus rhesus.—October 24, 1906, 3:30 P. M. After clear fluid was obtained, one cubic centimeter of a turbid suspension of Culture "654" was injected. The injected quantity was the equivalent of two agar slants; the culture had proved itself of relatively low virulence for guinea-pigs. October 25, 10 A. M., monkey sick; sits on perch holding head in hands; easily disturbed. L. p. no fluid obtained; needle-point covered with exudate consisting of leucocytes crowded with diplococci staining sharply. The nuclei of a small number of cells contained cocci. October 26, 12 M., monkey brighter and more active. L. p. slightly cloudy fluid flowed at once from the needle. C. s. showed leucocytes in good numbers, some of which contained few and others very large numbers of cocci staining feebly. Many of the leucocytes had probably recently emigrated since they were of normal appearance with circular (horse-shoe), non-fragmented nuclei. October 27, monkey recovered. November 10, animal in excellent condition.

November 14, 4 P. M., l. p. gave fluid readily; it flowed from needle as if under some pressure. One cubic centimeter of suspension of coccus "596" injected. No immediate effect produced. 6 P. M., monkey less lively. November 15, 7 A. M., found dead; evidently died only a short time before. Autopsy. The spinal membranes were distended and on incision a small quantity of turbid fluid escaped. The cord appeared pale, probably because of the turbid exudate which covered it. The convex surfaces of the brain were moist; definite inflammatory exudate was not visible; the basal membranes, from the medulla to the optic commissure, were infiltrated and distended with a turbid exudate. The pituitary body was surrounded with exudate; the ventricles were not widely dilated. The nasal mucosa was pale. Of the other organs only the lungs showed a pathological condition, namely, congestion and œdema. Cultures from the brain and cord, made at 4 P. M., gave growths of the diplococcus; the heart and lungs gave no growth. At 5 P. M. two segments of the medulla, about 0.5 centimeter thick,

[10] Westenhoeffer, Pathologisch-anatomische Ergebnisse der oberschlischen Genickstarreepidemie von 1905, *Klinisches Jahrbuch*, 1906, xv, 447.

were placed in sterile glass dishes which were enclosed in second dishes filled with cotton saturated with water. One was put at 37° C., the other at 2° C. At 10 A. M. next day, cover-slips and cultures were made; the latter gave no growth. C. s. showed the diplococci to have disappeared almost entirely from the tissue kept in the thermostat, the few remaining cocci being pale and ill-staining; and the diplococci from the second piece of tissue kept at 2° C. to be, perhaps, somewhat less numerous than in the control, but to stain sharply and well.

Histological examination of the central nervous system establishes the existence of a severe acute fibrino-purulent inflammation of the meninges of the cord and brain. The exudate in the cord is thicker over the posterior surface and it surrounds all the nerve roots; the dura mater is also invaded with pus cells. The exudate covering the medulla is abundant, while that covering the cortical surfaces is slighter in amount. The sulci show more exudate than the convexities. Two appearances should be emphasized: the great proliferation of endothelial cells of the pia, which cells mingle with the polynuclear leucocytes; and the diplococci, which are abundant in the exudate of the cord and medulla, innumerable in the exudate about the pituitary body and sparse in the exudate of the cortex and ventricles. The diplococci are, excepting those about the pituitary body, almost exclusively intracellular; and although a slight grade of encephalitis occurs, very few diplococci can be discovered in the leucocytes in the brain tissue. Thrombi of leucocytes occlude many veins.

The experiments reported thus far would seem to establish conclusively that the lesions and, to a certain extent, the symptoms of acute meningitis as they occur in man can be reproduced experimentally in monkeys. Since in the course of the experiments, the symptoms during life were observed incidentally only, the striking ones alone were recorded. Attention should be directed to the occurrence of nystagmus in a rhesus monkey which succumbed to inoculation. It should be stated, also, that not all the monkeys inoculated into the spinal canal succumb to the injections, next that after apparent recovery death may take place suddenly from causes which the post-mortem examination may fail to reveal, and finally that subcutaneous and intramuscular injections of large quantities of the diplococcus cause slight or no evident symptoms of disease. The cases of recovery will be illustrated by a few protocols. It should be noted that as recovery proceeds spinal puncture may show increasing cellular exudation into the canal and progressive degeneration of the diplococci. Cultures from the exudate at first positive become negative before all the diplococci disappear from it.

Monkey No. 5. Macacus nemestrinus.—May 15, 1905. This animal had been injected, April 12, 1905, with a purulent exudate without result (page 146). At

4:30 P. M. one cubic centimeter of a turbid suspension in salt solution of a diplococcus in its fifth generation from the spinal canal of case "Behren" was injected into the spinal canal. No symptoms had appeared by 9:30 P. M. May 16, 8 A. M., animal refused food and appeared sick. 11 A. M., had left perch and sat on bottom of cage. Appeared unable to lift himself on to the perch; while being observed two convulsions occured. Placed on the perch, he held on with hands and feet and was in a state of unstable equilibrium. Made no effort to escape from the cage on being handled. 10 A. M., lumbar puncture; a small amount of bloody fluid containing a moderate number of leucocytes and extra- and intracellular diplococci obtained. 3:30 P. M., l. p.: a small quantity of purulent fluid secured. The greater number of cocci were within leucocytes, many of which showed fragmented nuclei. Cultures from the exudate positive for the diplococcus. 6 P. M., back on perch. May 17, 8 A. M., again on bottom of cage; looked very sick, but took a little milk. 10 A. M., l. p.: the number of diplococci had diminished; very few were outside of cells; those in leucocytes stained feebly. May 18, 10 A. M., l. p.: a few drops of blood-tinted fluid secured; c. s.: some polymorphonuclear leucocytes, increasing number of mononuclear leucocytes, and a very small number of leucocytes containing degenerated diplococci; a few extracellular diplococci still present. Cultures negative. This monkey recovered rapidly.

Monkeys No. 19 and 20. Macacus rhesus.—June 27, 1905, 10 A. M. Each of these monkeys was injected with one third culture "Smith." They became very sick during the afternoon, and lay on the bottom of the cage. 10 P. M., still very sick. June 28, 9 A. M., both animals looked brighter. 10 P. M., No. 20 seemed livelier than No. 19. June 28, 12 M., l. p.: both yielded a small quantity of turbid fluid. The fluid from No. 19 and No. 20 contained many leucocytes; the fluid from No. 19 contained typical intracellular diplococci in small numbers, while that from No. 20 contained none. A culture was made from each fluid: No. 19 gave one colony of the diplococcus, No. 20 gave none. Complete recovery quickly followed in both instances.

Monkey No. 35. Macacus rhesus.—July 10, 1906, 10 A. M. Given 1 c.c. of thin suspension of Diplococcus "596." Spinal fluid obtained with syringe. The only symptoms which developed were slight depression, and erection of the hair covering the body. July 11, 10 A. M., l. p.: a small amount of whitish fluid from which the cells quickly subsided, was obtained. C. s. showed a large number of agglomerated, polymorphonuclear leucocytes, and very few diplococci, chiefly contained in the cells. The few free cocci could easily have come from broken-up leucocytes. At this time, a second injection of 0.5 c.c. of a similar suspension of Diplococcus "596" was made; no result. Animal quickly recovered.

To insure a fatal outcome a diplococcus of known virulence in a sufficient dose must be injected. Individual differences in susceptibility occurred among the monkeys, but no monkey was wholly refractory to inoculation. As compared with the doses which probably determine infection in man those used to produce the experimental disease in monkeys are colossal. As an example of

refractoriness the next experiment will serve; it will show also how small the effect produced by subcutaneous and intramuscular injections of the diplococcus may be.

Monkey No. 26. Macacus rhesus.—October 28, 1905, 12:30 P. M. One "Smith" culture injected. 3:30 P. M., l. p.: turbid fluid flowed from the needle. C. s. showed many diplococci and very few leucocytes. Some of the cocci grouped about the leucocytes. October 29, 12 M., l. p. not successful; one half culture "Smith" injected (probably into muscles of back). October 30, 3:30 P. M., l. p. unsuccessful. Injected two cultures "Smith" (probably into muscles of the back). 6 P. M., animal slightly depressed; off perch. October 31, A. M., animal active. 3 P. M., l. p. yielded with difficulty a small amount of bloody fluid containing leucocytes and few diplococci. Injected three cultures "Smith"; no effect. This monkey showed remarkably little depression following the injections, and was soon in a normal condition.

It is somewhat remarkable that the later injections failed to influence the result of the first injection, which evidently reached the spinal canal. I have observed many other examples of the relative innocuousness of the diplococcus when introduced beneath the skin and into the muscles. The effects of large doses given in this way may be nil, or a slight elevation of temperature may appear and remain for a few hours, or the animal show for a brief period disinclination for food. The next experiment is introduced as an example of sudden death, after apparent recovery from an inoculation.

Monkey No. 21. Macacus rhesus.—July 1, 1905, 10 A. M. Two thirds culture "Smith" injected. No fluid obtained prior to injection. No symptoms developed, but the canal must have been entered as lumbar puncture July 2, 12 M., yielded a small quantity of turbid fluid containing leucocytes and very few degenerating, intracellular diplococci. No growth was obtained on sheep-serum agar. No symptoms of disease were noted in this animal. July 6, found dead. At the autopsy more clear fluid than is usual escaped from the dura surrounding the cord. The lower segment of the cord was congested. No exudate was visible and the ventricles were not dilated. The pial vessels of the medulla were injected. Smear preparations from the spinal cord and several parts of the brain showed neither diplococci nor polymorphonuclear cells. The sections of the tissue show no inflammation or leucocytic infiltration except superficially in the lumbar region in the posterior columns of the spinal cord at what was probably the point of entrance of the needle.

In contrast to the ease with which the acute inflammations can be excited is the difficulty which attends the production in monkeys of a subacute form of meningitis. The intraspinal injections pro-

duce either an acutely fatal meningitis, or an acute disease from which recovery takes place rapidly. The animals which survive inoculation are usually well of the disease in three or four days. In rare instances, the animal lingers a victim of the disease for a longer period than this to succumb in the end. Monkeys which survive the second day after inoculation tend rather to recover than to die. By following in inoculated monkeys the changes in the cerebro-spinal canal by means of lumbar puncture, the progress of the infection can be traced and the result often predicted. Disappearance of the diplococci early from the canal is a good sign; early emigration of leucocytes is also a good sign; early emigration, active phagocytosis and dissolution of the diplococci, both within and without leucocytes, are very favorable signs. Proceeding in this way, I have been able to keep a small number of monkeys in a state of subinfection with the diplococcus for a period of several days or weeks. The period of successive inoculation was determined by the puncture and by the physical condition of the monkey. A state of resistance tended to develop which necessitated the employment of increasing amounts of cultures to produce visible effects; and in the end the run-down animal succumbed to the dose administered. In these animals the exudates were thicker and firmer and covered the base and the convex surface of the brain; and the ventricles were sometimes widely dilated and contained turbid exudates. The first of these experiments was made with a South American species of which the next protocol gives an account.

Monkey No. 27. South American; Genus Cebus.—October 7, 1905, 11 A. M. Injected suspension of one agar culture "Whitaker," three days old. No symptoms developed. October 9, 11 A. M., l. p.: free flow of turbid fluid, of which 1 c.c. was collected. Injected one "Smith" culture eighteen hours old. 1 P. M., animal crouched in corner; head down and resting in hands; indisposed to move. 5:30 P. M., condition about the same; l. p.: a small quantity of fluid containing leucocytes, but no diplococci obtained. Culture negative. October 12, 3 P. M., l. p. yielded a small amount of turbid fluid containing vacuolated leucocytes and mononuclear cells; no diplococci. One half agar slant culture of "Smith" injected. 9:30 P. M., animal depressed. October 13, animal on perch. October 16, appeared to be well. Suspension of two old "Smith" cultures injected. October 17, no marked effects. L. p. gave a few drops of faintly turbid fluid showing leucocytes with intact nuclei, of which some contain degenerated diplococci. October 18, l. p.: faintly turbid fluid flowed freely from the needle. C. s.: leucocytes and

mononuclear cells; no cocci. A number of vacuolated (or fatty) large mononuclear cells present. Injected one twenty-four hour culture "Smith." No symptoms developed. October 20, 3 P. M., l. p. gave small amount of turbid fluid showing leucocytes but no cocci. Culture negative. Injected one culture "Smith" twenty-four hours old. No symptoms. October 23, l. p.: 0.5 c.c. turbid fluid obtained containing leucocytes and a few red corpuscles. October 28, l. p. gave clear fluid containing mononuclear cells. Injected two "Smith" cultures. October 30, l. p.: a drop of turbid fluid obtained showing leucocytes with fragmented nuclei but no cocci. Injected two "Smith" cultures. October 31, l. p. yielded a few drops of turbid fluid showing many leucocytes, and large mononuclear cells; no definite cocci; culture negative. During this treatment the monkey lost in weight. No further injection was given until November 15, at 12:30 P. M., at which time a heavy suspension of a "Smith" culture, eighteen hours old (representing four agar slant surfaces) was injected. Symptoms rapidly developed; at 2:30, animal sick; l. p. gave with difficulty a few drops of blood-tinted fluid containing many agglutinated diplococci and a small number of leucocytes, of which some had included diplococci. 4 P. M., l. p. gave, with difficulty, little fluid in which diplococci were numerous. October 16, 9 A. M., monkey died. Autopsy. No excess of fluid in the meninges of the spinal cord, and no exudate was visible. Turbid fluid, in excess of the normal, infiltrated the meninges from the medulla to the optic commissure. The anterior and lateral pial surfaces of the hemispheres showed smaller and larger ecchymoses. The ventricles contained small quantities of turbid fluid; they were not dilated. Cultures from different parts of the brain and cord remained sterile. The cover-glass preparations from different levels of the spinal cord, the base and convexity of the brain, choroid plexus and ventricles differed from one another only in minor details. Several striking conditions were shown by them to exist in the membranes. In the first place, no, or almost no, normal cocci remained, but only fragments of swollen and degenerated cocci which were entirely or nearly so within cells. The cells consisted of polymorphonuclear leucocytes which predominated in number and contained the far greater number of cocci; and of much larger cells of wholly different aspect. The latter were several times as large as the leucocytes, and occasional examples were of colossal size (equal to 20 to 40 leucocytes). The nucleus was single, as a rule, and oval or crescentic in form, and vesicular. The protoplasm was pale, and the membrane at the periphery stained deeply. The smaller of these cells contained at times, degenerated cocci; and all may have ingested a variable number of ordinary leucocytes, or mononuclear cells. They were most numerous in the smears prepared from the exudate of the meninges of the medulla. The sections of the central nervous system present quite a different appearance from the other cases described. Although the exudate is thicker than in the previous instances, the chief novelty lies in the great proliferation of the fixed cells of the pia-arachnoid. The multiplication has taken place chiefly along the surface of the brain and at the outer line of the membrane. The new cells are relatively large and vesicular, and their occurrence in double rows gives at times a somewhat glandular appearance to the membrane. Polymorphonuclear leucocytes are numerous and distributed irregularly among the other cells. The degree

of encephalitis is far less than in many of the experiments. The main arterial branches of the brain show a more or less marked infiltration of the intima with leucocytes, and leucocytic invasion of the sub-intimal and inner-muscular layers of the vessels.

This experiment brings out the remarkable capacity which can be developed in the monkey to dispose of cultures of the diplococcus injected into the spinal canal. It is shown by the experiments that the actively bactericidal cells and secretions of the canal are capable of destroying, in a few hours, prodigious numbers of the diplococcus, and that agglutination of the diplococcus can also take place in the canal. Finally, it is shown that the monkey can be killed by an overdose of culture which causes death by poisoning and not by infecting the animals directly; for the lethal effect occurred in this case, although the diplococci were themselves quickly killed. Increased power, therefore, to destroy the diplococcus is not associated necessarily with an equally increased power to resist the toxic effects of the intracellular poison. This fact must have an important bearing upon a specific therapy of diplococcus meningitis.

The experiment is important in respect to the histological alterations present in the meninges. In no previous experiment did the exudate contain so large a number of cells which differed from leucocytes. The large cells described here occur regularly in cases of human meningitis and have been frequently observed. Their occurrence in numbers in this monkey serves to knit even more closely the experimental and the human diseases. Flexner and Barker,[11] and Councilman, Mallory and Wright[12] describe and figure these large cells among the leucocytic infiltration, and the latter investigators have traced their origin to the cells of the connective tissue and the lymph spaces of the pia-arachnoid. The histological conditions present in the membranes are comparable with those observed in cases of meningitis of longer duration in man.

Monkey No. 32. Macacus rhesus. April 19, 1906, at 12 M., given one agar culture "548" in the third generation. 3 P. M., l. p., no fluid obtained. April 20, 12 M., l. p., small quantity of clear fluid containing few leucocytes and free cocci. 6 P. M., animal depressed. April 21, active. April 22, 12 M., injected

[11] A contribution to our knowledge of Epidemic Cerebro-Spinal Meningitis. *Amer. Jour. of Med. Sciences* 1894, CVII, 155, 259.

[12] *Op. cit.*, p. 102.

one culture; no apparent effect. April 23, 4 P. M., l. p., very little fluid obtained. April 25, 12 M., one agar culture "Gratz." Fluid obtained before injection which produced no visible effect. April 26, one agar culture "Gratz." No symptoms. May 1, 3 P. M., l. p., yielded slow flow of faintly turbid fluid showing leucocytes and larger cells but no cocci. Injected turbid suspension (equal to four agar cultures). 6 P. M., animal sick. Died during the night, May 2. Autopsy: The spinal membranes contained an excess of fluid. On opening the dura mater a gelatinous exudate was seen to occupy the meshes of the pia. A similar exudate covered the base of the brain and surrounded the foramen of Magendie. The convex surface of the brain was greatly altered by a moderately firm and adherent exudate of white color which occupied the pia-arachnoid. It covered the surface of the convolutions and filled the sulci, thus giving a remarkably smooth appearance to the brain (Plate IV, Fig. 6). The ventricles were considerably dilated and contained an excess of turbid fluid. Smear preparations showed the chief accumulation of leucocytes to be at the base of the brain and the lower portion of the spinal cord. The lateral surfaces of the brain showed fewer and more were present in the choroid plexus than in the fluid of the ventricles. Diplococci were present everywhere; they were intracellular and often degenerated. The number varied: they were abundant at the base and less numerous at the convexity of the brain. The mucous membranes of the nose showed, among the columnar epithelium, a number of fragmented leucocytes carrying diplococci indistinguishable from those of the brain (Plate V, Fig. 7). Sections of the spinal cord and brain bear out the findings described. The exudate occurs throughout the membranes covering these structures. It is thicker over the posterior than over the anterior surface of the cord, and while it is abundant over the convolutions, it is especially thick in the sulci. Little or no fibrin occurs. The exudate consists chiefly of polymorphonuclear leucocytes among which there are a large number of moderately large mononuclear cells. The brain substance is remarkably free from infiltration, while the lumbar cord shows invasion, about its posterior surface, to a considerable depth. The dura of the cord at certain levels shows leucocytic infiltration. Diplococci which are very numerous in the exudate, are almost entirely within leucocytes. They have been carried by these cells into the substance of the spinal cord.

A striking feature of the sections is derived from the width of the ventricles. As a rule, these appear as slits in the sections; in this case they are wide cavities. Usually, the ependymal epithelium is regular and relatively high; in this case, it is often depressed or flattened, and a considerable flattening of the choroid plexus, toward the wall of the ventricle, is noticeable. A considerable degree of sub-epithelial cellular proliferation has taken place in the walls of the lateral and fourth ventricles. Leucocytes are moderately abundant in the ventricles.

The dura mater was removed with its attachments to the ethmoid bone, and longitudinal sections prepared. The leucocytes surround the olfactory bulbs in dense masses, and they push along clefts and spaces in the dense membrane, existing now as elongated columns and now in circumscribed, abscess-like masses.

This experiment is valuable in showing that a condition of subacute infection of the meninges can be procured in the monkey by

the use of extraordinary means, and when the condition is present and the thick exudate incidentally covers and occludes the foramen of Magendie, a degree of internal hydrocephalus develops. The experiment enforces a point already made. The histological study of the dura mater at the anterior extremity shows that leucocytes pass into its substance for a considerable distance beyond the proper confines of the cranial cavity. The cells could, indeed, easily carry diplococci with them, in which case an explanation for the occurrence of leucocytes carrying diplococci in the nasal mucosa might be readily found.

The series of experiments described suffices to establish the varieties of effects which are produced upon the central nervous system of lower monkeys by means of intra-spinal injections of cultures of *Diplococcus intracellularis*. Among the histological appearances mentioned, there was one monkey, No. 17, which deserves more particular attention. I described, in this case, a condition of acute endarteritis of the type frequently found in cases of human meningitis. It would appear that this pathological condition occurs in many forms of meningitis as is shown by the investigations of Hektoen,[13] Councilman, Mallory and Wright,[14] and others. The latter authors state, indeed, that it is less commonly found in the epidemic (*Diplococcus intracellularis*) form of meningitis than in other forms (pneumococcus, etc.) of the disease. They figure a slight degree of the condition in a goat in which they produced experimental meningitis by injecting the diplococcus. I have encountered in still another monkey inoculated with the diplococcus very extensive acute endarteritis. The case will be described in full in my article on a serum therapy for experimental meningitis. A spider monkey (*Atelas ater*) was inoculated on December 2, 1905, at 12 o'clock noon, with a slant agar culture of the diplococcus. At 3 P. M. two cubic centimeters of anti-goat (diplococcus) serum were injected into the spinal canal. Death occurred December 3, 11 A. M. The lesion mentioned affects arteries of all sizes in the brain. At the base, over the medulla, rarely does

[13] The Vascular Changes of Tuberculous Lepto-meningitis, especially the Tuberculous Endarteritis, *Jour. Exp. Med.*, 1896, I, 112.

[14] *Op. cit.*, p. 77.

an artery escape. The arteries of the convexity are affected less uniformly, and those of the spinal cord least of all. The systemic arteries do not show the lesion. The subendothelial infiltrations of mononuclear and polynuclear cells may or may not occlude the vessel entirely (Plate V, Fig. 8). Both large and small vessels may be partially or wholly filled up with cells which, as a rule, push the endothelium before them. The endothelium is preserved unless lost from mechanical causes in making the sections. An appearance has been obtained in some vessels of a mushroom-like overlying of the endothelium by the new cells which have accumulated in the adjacent part of the vessel. The muscular coat is usually free from infiltration, as are the veins. This monkey showed an unusual degree of hæmorrhage into the meninges, and definite cortical abscesses, of small size, extending into the brain from the cortical membranes, or developing about the intra-cortical blood-vessels. The number of diplococci in the inflammatory exudate was not large.

The pathological effects and appearances which have been described are not, probably, specific. The experiments are, therefore, valuable, not because they show that *Diplococcus intracellularis* alone of the pyogenic micrococci can produce acute meningitis in monkeys, but because they prove that the diplococcus is capable of setting up in these animals lesions of meningitis which bear a close resemblance to the lesions which the same micro-organism causes naturally in human subjects. The finer the details of correspondence are between the natural and experimental disease, the more valuable do the experimental results become, and the more convincing are they that *Diplococcus intracellularis* is really the cause of epidemic meningitis in man. I have not undertaken to study all the common pyogenic micrococci in the manner of this study of the diplococcus, but I have made, for obvious reasons, a single experiment with *Micrococcus catarrhalis*. The culture employed was kindly given me by Dr. Elser. It fulfilled the usual biological tests for that micro-organism.

Monkey No. 28. South American; Genus Cebus.—October 12, 1905, at 3 P. M., inoculated three agar slant cultures of *M. catarrhalis*. Clear fluid flowed from the needle before the injection. 9 A. M., animal sick; sat on

bottom of the cage. October 13, 9 A. M., condition unchanged. 3 P. M., very sick; head rested on hands. L. p., 9 A. M., yielded a very small quantity of fluid by using syringe. C. s., many leucocytes containing intracellular cocci in moderate numbers; a few cocci extra-cellular. The cocci appeared larger than the diplococcus; some stained feebly. October 16, animal had lost flesh but was recovering. Sat on perch. L. p., 3 P. M., with the syringe a few drops of faintly turbid fluid obtained. It showed small numbers of leucocytes with fragmented nuclei, but no cocci. This animal never fully recovered its flesh, and the hair entirely disappeared from the long tail. It was found dead on February 12, 1906. The autopsy showed the brain and cord to have returned, apparently, to the normal condition. Cover-slips were free of leucocytes and cocci; cultures remained sterile. An adequate cause of death was not found. Nearly all of this species of monkey contain adult filaria in the peritoneal cavity, and filarial embryos in the blood.

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By means of the experiments recorded in this paper, it has been shown that the lower monkeys can be infected without great difficulty with *Diplococcus intracellularis* and made to reproduce the pathologic conditions present in man in cerebro-spinal meningitis. The experiments establish that the diplococci, when introduced in a low level of the spinal canal, distribute themselves in a few hours through the meninges and excite an acute inflammation, the exudate of which accumulates chiefly in the lower spinal meninges and the meninges of the base of the brain. The uniformity with which the chief exudate is found at the base of the brain and the rarity of its appearance in equally great amounts over the convexity is a fact of importance in the dynamics of the cerebro-spinal fluid since there is relation between the quantity of exudate and the accumulation of the diplococcus. This distribution of the exudate led me to doubt the validity of the reasoning which would ascribe this localization of the inflammation in man to the entrance into the meninges of the infective agent directly through the nasal membrane. This tendency to localization at the base of the brain in monkeys is especially interesting in view of the fact that it is usually only a short time before death that they lie down upon the side of the body. In comparing the experimental lesions in monkeys with the naturally developed lesions in man, note should be taken of the occurrence under both conditions of encephalitis and abscesses, of hæmorrhages, of proliferation of large cells of connective tissue and tissue spaces, of acute endarteritis, of inflammation of the dorsal

root ganglia, of internal hydrocephalus, of relatively small amount of fibrin in the exudates, of fibrinous and other thrombi, and of phagocytosis of diplococci and somatic cells.

The inflammation of the meninges extends in monkeys into the membranes covering the olfactory lobes and along the dura mater into the ethmoid plate and nasal mucosa. The nasal mucous membrane is found, in many instances, to be inflamed and beset with hæmorrhages. Smear preparations from the nasal mucous membrane, from the higher levels especially, have shown me polymorphonuclear leucocytes, often in numbers, carrying diplococci which presented the form, size, staining qualities and degenerations of the diplococci occurring in the same cases in the cerebral and spinal meninges. Thus far *Diplococcus intracellularis* has not been cultivated from the nose of the infected monkeys. I have, however, secured other Gram-negative diplococci with which, it may be stated, the intracellularis could not be confounded.

Although the pathological effects produced in monkeys are comparable with those occurring in the natural disease in man, there is no real correspondence in the relative degree of susceptibility of the two species. The quantity of an active culture required to cause marked symptoms or to bring about death from meningitis in monkeys, is prodigious if compared with the number of diplococci which probably suffice to produce infection in human beings. Moreover, the amount of multiplication of the diplococcus in monkeys, excepting possibly in the focal abscesses, is under the most favorable conditions, small; and I am not disinclined to believe that in many of the experiments no multiplication whatever took place.

The experiments, the details of which have been set down here, besides being an argument for the causative action of *Diplococcus intracellularis* in epidemic meningitis, form, also, the basis of an attempt to influence the progress and termination of the experimental infection in monkeys through the employment of anti-sera prepared from the diplococcus. The results of the experiments with the anti-sera are given in a separate article.

I wish to acknowledge the valuable aid rendered by my former assistant, Dr. H. S. Houghton, in the course of these experiments.

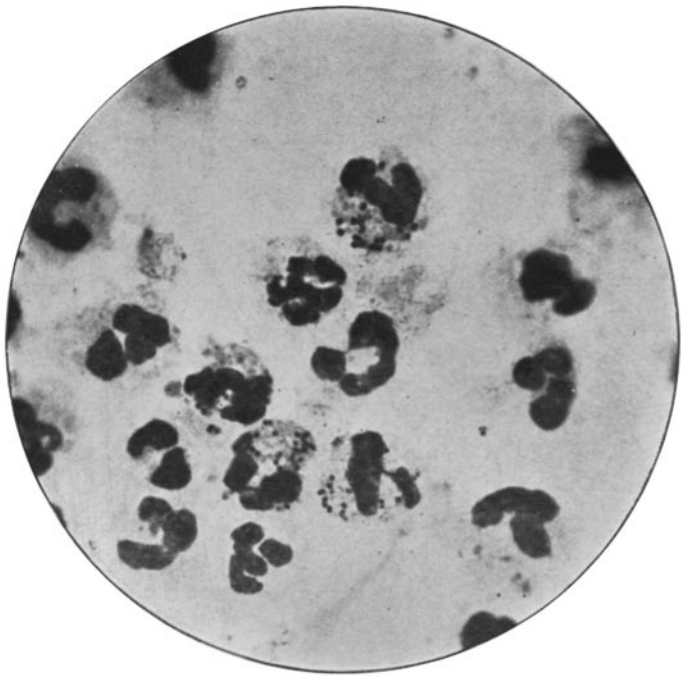

FIG. 1.

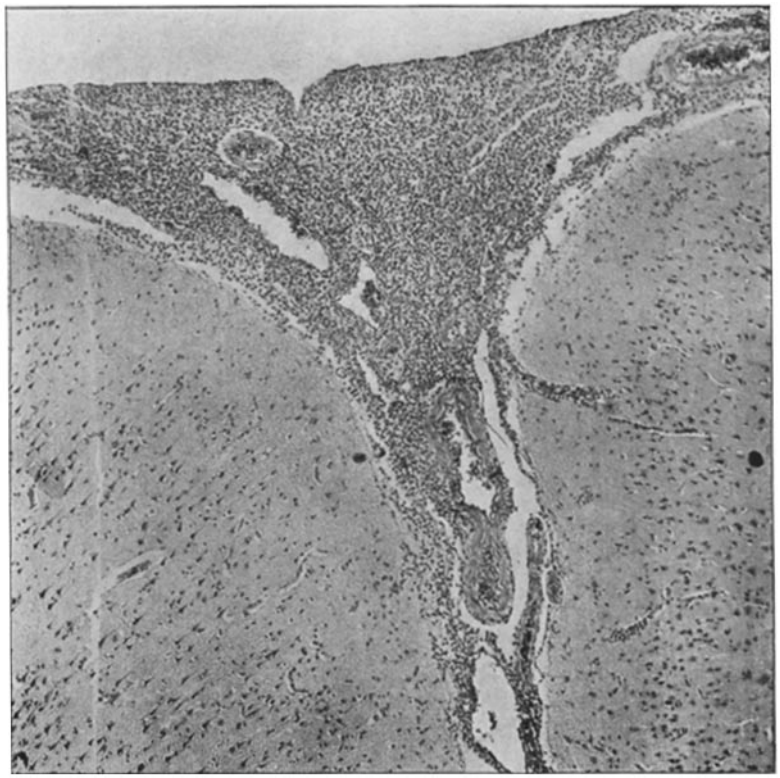

FIG. 2.

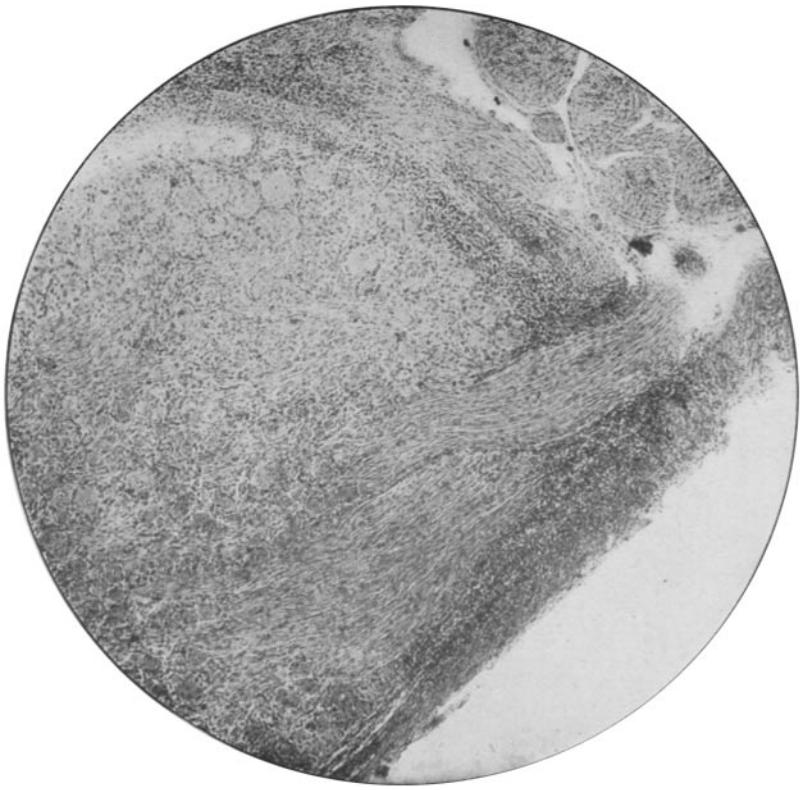

FIG. 3.

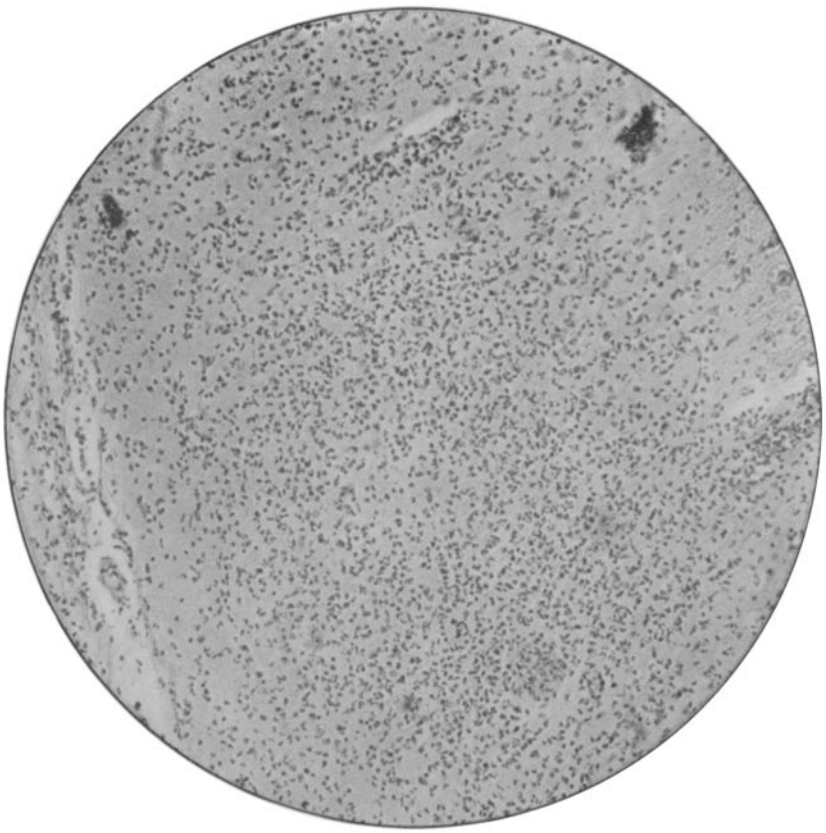

FIG. 4.

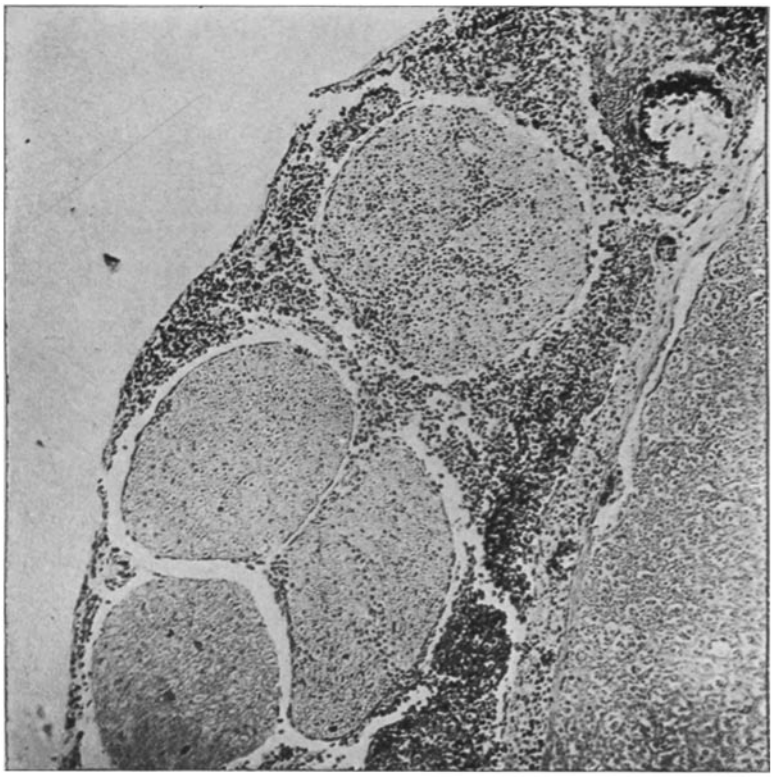

FIG. 5.

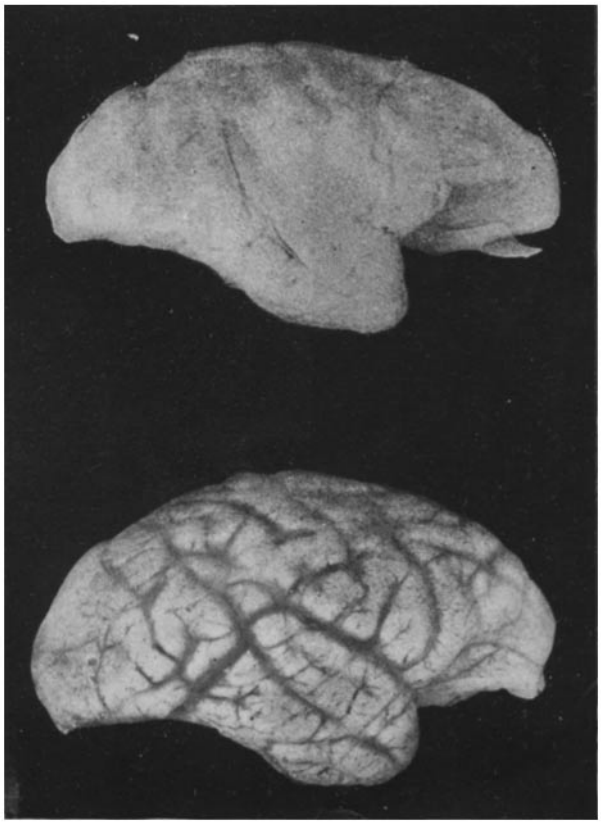

FIG. 6.

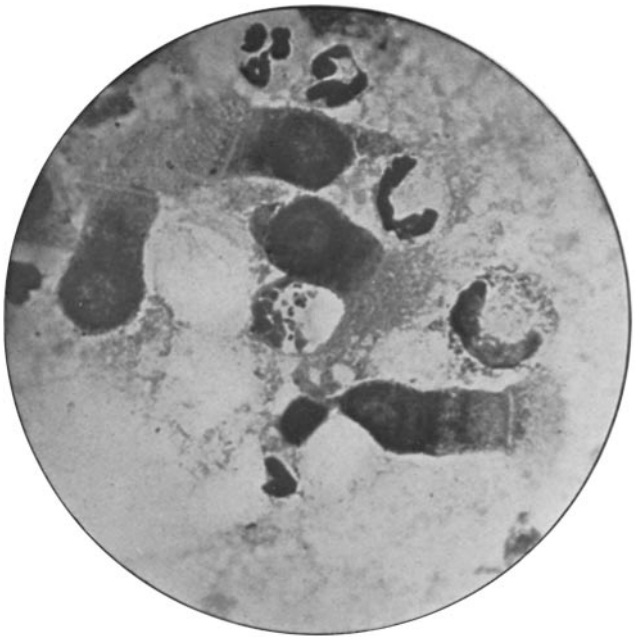

FIG. 7.

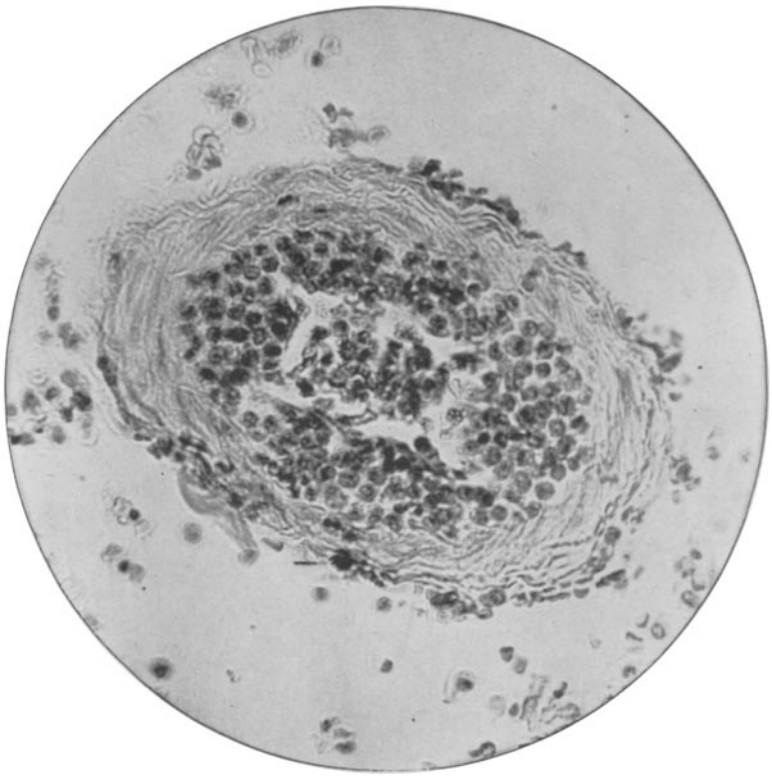

FIG. 8.

DESCRIPTION OF PHOTOGRAPHS.

PLATE II.

FIG. 1. Smear preparation from contents of brain abscess, monkey No. 1. Typical intracellular diplococci. Methylene-blue staining. $\frac{1}{12}$ in. objective.

FIG. 2. Low magnification to show the relation of the exudation in the pia-arachnoid of the convex surface of the brain and the sulci, and the extension of the exudation along the cortical vessels into the brain.

PLATE III.

FIG. 3. Spinal ganglion showing leucocytic invasion about and between the nerve-cells of the ganglion.

FIG. 4. Low magnification to show the degree of acute encephalitis, and the involvement of intracortical blood-vessels in the infectious processes.

PLATE IV.

FIG. 5. Low magnification to show the exudation about and within the spinal nerves.

FIG. 6. Brain, natural size, from a case (Monkey No. 32) of subacute meningitis compared with the brain from a case of very acute experimental meningitis in which the congestion of the pial vessels is very marked.

PLATE V.

FIG. 7. Smear preparation from inflamed nasal mucosa showing a leucocyte, partly broken, enclosing diplococci, lying among columnar ciliated epithelial cells.

FIG. 8. High magnification of an artery of the base of the brain showing the lesion of acute endarteritis.

I am indebted to the skill and kindness of Dr. Edward Leaming for the photographs illustrating this paper.